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Original Article

Type 1/Type 2 Cytokine Serum Levels and Role of Interleukin-18 in Children with Steroid-Sensitive Nephrotic Syndrome

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Abstract

Introduction: In view of the conflicting evidence of helper T cell type 1 (Th1) or type 2 (Th2) pattern of cytokine synthesis in steroid sensitive nephrotic syndrome (SSNS), this study aimed to assess type-1/ type-2 cytokines level in different stages of SSNS and to evaluate the role of IL-18.

Methods: We prospectively studied thirty children with SSNS, aged 2–12 years. The children were evaluated in the active stage before treatment initiation and re-evaluated again during remission while still on steroid treatment. A subgroup of children (21/30) was also evaluated during remission after steroid withdrawal. The control group included 30 healthy age- and sex-matched controls. Serum levels of IL-2, IFN-γ, IL-4, IL-13 and IL-18 were measured by ELISA.

Results: IL-2 levels were not significantly different between children in different stages of SSNS and controls ($p > 0.05$). Levels of IL-4, IL-13 and IL-18 were significantly higher during the active stage of SSNS compared to remission and controls ($p<0.05$). Serum IFN-γ was significantly lower in children with active disease compared to remission stages and controls ($p<0.05$). In children with SSNS, serum levels of IL-18 correlated significantly with both IL-4 and IL-13 during all stages ($r = 0.72$ and $p<0.0001$, $r = 0.82$ and $p<0.0001$, respectively).

Conclusion: Children with active SSNS seem to have a shift to type-2 cytokine production, and IL-18 expression is significantly correlated with this type-2 immune response.

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Introduction

Idiopathic nephrotic syndrome (INS) is considered to be an immune-mediated disease [1]. How T-cells affect the course of the disease remains an unanswered question. However, circulating factors were proposed to be released from activated T-cells which may affect the pathogenesis of the disease. Several cytokines are considered prime candidates for the role of mediators of INS [2].

Based on the predominant cytokines, the immune response can be functionally subdivided into type-1 and type-2. Type-1 response normally prevails. It produces interferon-gamma (IFN-γ) and interleukin (IL)-2 and enhances the production of complement-fixing and opsonizing antibodies as well as macrophage activation resulting in delayed type hypersensitivity (DTH) response. Type-2 response is dominated by IL-4 and IL-13 and provides optimal help for antibody production. It promotes both mast cell growth and eosinophil differentiation and activation, resulting in humoral responses [3].

Measurement of cytokine level in serum or urine in INS patients has been performed by many investigators. Increased levels in serum or urine in relapse were reported for interleukin (IL)-2 [4], soluble IL-2 receptor [5-8], and (IFN-γ) [5, 9], IL-4 [9, 10], IL-12 [11]. INS has been suggested to be a Th2-dependent glomerular disease [12] depending on the evidenced association between it and atopy and allergy [13-15]. Many allergic disorders such as asthma, allergic rhinitis and eczema, are typically linked to the presence of a Th2 immunologic response. Further support to the hypothesis that INS is a Th2-dependent glomerular disease comes from the elevated serum IgE and preservation of IgG4 observed in INS

[16-18]. In addition, levels of IL-4 and IL-13, which are two of the major Th2-associated cytokines, have been shown to be elevated in INS patients in relapse [9, 10, 16, 19]. However, contradictory observations have been also reported [19- 21].

IL-18 is an exclusive cytokine which can stimulate both type-1 and type-2 immune responses depending on the cytokine environment [4]. Since Matsumoto and Kanmatsuse [22] have demonstrated that IL-18 was involved in the active stage of the disease and that there was a positive correlation between IL-18 production and disease activity, efforts have been made to identify the pathogenetic vascular permeability factors VPF(s) released from T-cells, such as cytokines, as well as to clarify the participating cells in its pathogenesis. For the last two decades many studies investigated the cytokine pattern in steroid sensitive nephrotic syndrome (SSNS) with sometimes conflicting results [5–7]. Besides, there is paucity of literature regarding the role of IL-18 in disease process. The current study aimed to evaluate type-1/ type-2 cytokine profile in children during different stages of primary SSNS and to investigate the involvement of IL-18.

Methods

This is a prospective longitudinal study carried out in Madinah Maternity and Children Hospital (MMCH), the main maternity and children referral hospital in Al-Madinah AI-Munawarah, KSA. The study investigated thirty children with proven first episode of steroid-sensitive nephritic syndrome (SSNS) who were admitted in our department during the period from August 2010 to August 2012. Active stage of SSNS was defined as increased urinary protein excretion (Albustix ≥2+ for at least three consecutive days or > 40 mg/m^2/hour) and serum albumin ≤2.5 g/dL. Remission was defined as serum albumin concentration ≥ 3.5 g/dL and normal protein excretion (Albustix trace or negative for at least three consecutive days or ≤ 5 mg/m^2/hour) [23]. Children with SSNS were evaluated during the active stage of the disease and during remission while still on steroids at a dose of 40 mg/m^2 on alternate days. Twenty-one children were evaluated again during the remission phase after being off steroid treatment for at least 6 months. No patient was taking immunomodulating drug(s) nor did they have history of recent (within the previous 6 months) infection and/or inflammatory conditions, or abnormal urinary sediments (casts or crystalluria). The control group included thirty age- and sex-matched healthy children who had come to the outpatient Hematology Clinic in order to be tested for β-thalassemia trait and were found to be negative. They aged 2-12 years (15 males and 15 females) and had urinary protein/ creatinine ratio less than 0.2 and serum albumin level of 4.1±4.6 mg/dL.

The study was approved by the Ethics Committees of MMCH. The parents of all patients and controls were informed about the study and gave their written consent. Blood samples were collected from patients and controls in test tubes without anticoagulant under sterile conditions. Serum was separated by centrifugation at 300g for 10 min, then divided in small aliquots and stored at −80^{o}C for future serum cytokine assessment. Serum IFN-γ, IL-2, IL-4, IL-13 and IL-18 concentrations were measured by using quantitative colorimetric sandwich ELISA kits (R&D China Co. Ltd., Shanghai). Following the manufacturer's instructions, each cytokine sample was run in duplicate and the mean cytokine concentration was calculated.

Statistical analysis was performed using Statistical Package for Social Sciences version 17, (SPSS Inc., Chicago, IL, USA). Values were expressed as medians and ranges. The non-parametric Wilcoxon signed-rank test was used to compare differences between study groups with paired data. For non-paired data, statistical significance was analyzed by the Mann–Whitney U test. Spearman's coefficient of correlation (r) and regression analysis model were used to determine the correlations. P value < 0.05 was considered to be statistically significant.

Results

The study investigated 30 children (17 males and 13 females) in the active stage of SSNS, aged 2 to 12 years (median = 3.52 years). The control group consisted of 30 healthy siblings of the patients (15 males and 15 females) aged 2 to 12 years (median = 4.12 years). There was no statistically significant difference between the group of children with nephrotic syndrome and the control group in regard to age or sex ((p > 0.05). Table-1 summarizes the results of serum IL-2, IFN-γ, IL-4, IL-13 and IL-18 levels and p values in all study groups. There was no significant difference in IL-2 levels between nephrotic children in all disease stages and controls (p > 0.05). Children in the active stage had significantly lower levels of IFN-γ when compared with the two remission phases (remission on steroids and remission off steroids (p = 0.005, p = 0.001, respectively) and the controls (p = 0.007), but there was no significant difference between controls and each of the two remission phases (p > 0.05). Children in the active stage of SSNS had significantly higher levels of IL-4 when compared with the two remission phases (p < 0.0001). Patients in both remission phases

Tabl-1: Serum levels and median and range values of IL-2, IFN-γ, IL-4, IL-13 and IL-18 in patients during different stages of SSNS and in controls

Serum cytokine levels	Active state (n = 30)	Remission on steroids (n = 30)	Remission off-steroids (n = 21)	Control Group (n = 30)
IL-2(pg/mL)				
Median	8.3	9.8	9.1	8.7
Range	8- 13.5	7.9- 12.8	7.6 – 12.9	7.9 – 13.1
(IFN-γ) (pg/L)				
Median	16.25*	24.5	25.9	21.8
Range	14.76 – 29.9	13.72 – 36.9	15.28 - 35.7	13.4 – 33.3
IL-4 (pg/mL)				
Median	65*	33.7†	21.9†	14.5
Range	19.7 – 109.5	15.8 – 81.8	11.6 – 69.5	7.7 - 42.
IL-13 (pg/mL)				
Median	59.9*	28.7†	19.7†	12.7
Range	32.5 – 160.8	11.8 – 133.7	11.1 - 96.3	10.4 – 20.6
IL-18 (pg/mL)				
Median	1564*	1311†	765†	109
Range	532.1 - 2716	417 - 2245	321 – 1278	29 - 236

* Significantly different from levels in remission and controls

† Significantly different from controls

showed significantly elevated IL-4 levels compared to controls ($p < 0.0001$ and $p = 0.034$, respectively). Serum IL-13 levels were significantly higher in the active stage of SSNS compared with the two remission phases ($p < 0.0001$). IL-13 levels were still elevated during both remission phases compared with the controls ($p < 0.0001$ and $p = 0.002$, respectively). In the active stage of SSNS, IL-18 levels were significantly higher compared with the two remission phases and controls ($p = 0.003$, $p = 0.001$, and $p<0.0001$, respectively). IL-18 levels in both remission phases, although lower than their levels in the active stage, remained significantly higher than controls ($p<0.0001$).

As shown in Figure-1, linear regression analysis revealed that serum IL-18 and IL-4 levels were significantly correlated in nephrotic children in all disease stages ($r = 0.72$ and $p<0.0001$), including the active stage of the disease ($r = 0.73$ and $p = 0.001$). Figure-2 illustrates the significant correlation between serum IL-18 and IL-13 levels in nephrotic children in all disease stages ($r = 0.82$ and $p < 0.0001$) as well as in active stage of the disease ($r = 0.76$ and $p = 0.001$).

Discussion

Studies of the type-1/type-2 cytokine patterns in the sera of patients with SSNS have generally been variable and inconsistent. These conflicting results may be due to the differences in the immunologic techniques used to assess cytokine synthesis [24]. Our study has clearly shown that children with active SSNS showed down-regulation of type-1 immune response. IL-2 serum levels were not significantly different in children with SSNS in all stages. This is in agreement with pervious cited studies [16–19], including the study by Printza *et al* [24]. On the other hand, Zachwieja *et al* [16] demonstrated the presence of higher intracellular expression of IL-2 using a three-color flow cytometric assay. Considering the physiological fact that increased intracellular production does not usually result in increased secretion, we can partially explain the difference between our results and Zachwieja *et al* [25].

In the group of children with active disease, IFN-γ serum levels were lower than both remission groups as well as controls. Our results are in accordance with the results of two previous studies [24, 26] which showed a decreased production of IFN-γ by stimulated peripheral blood mononuclear cells in patients with active SSNS. Cheung *et al* [27] and Kaneko *et al* [28], who assessed the percentage of IFN-γ producing N cells in patients with SSNS, did not find significant difference between patients and controls. The predominance of type-2 immune response in our children with active SSNS is further supported by the up-regulation of the serum levels of IL-4. These results are in agreement with earlier published works by Stokowski *et al* [20], Neuhaus *et*

Figure-1: Correlation of serum IL-18 and IL-4 levels in children with steroid sensitive nephrotic syndrome (SSNS) in all stages (a) and in children with active disease (b)

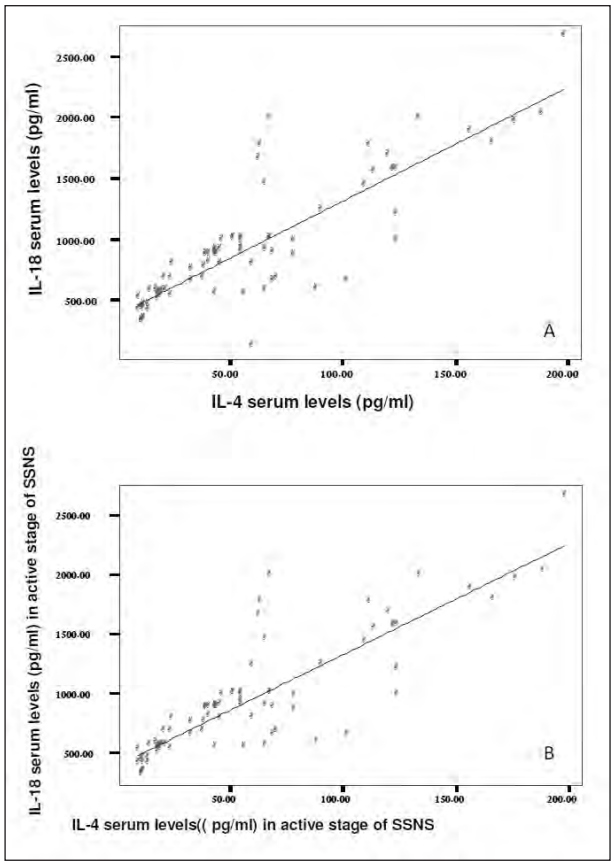
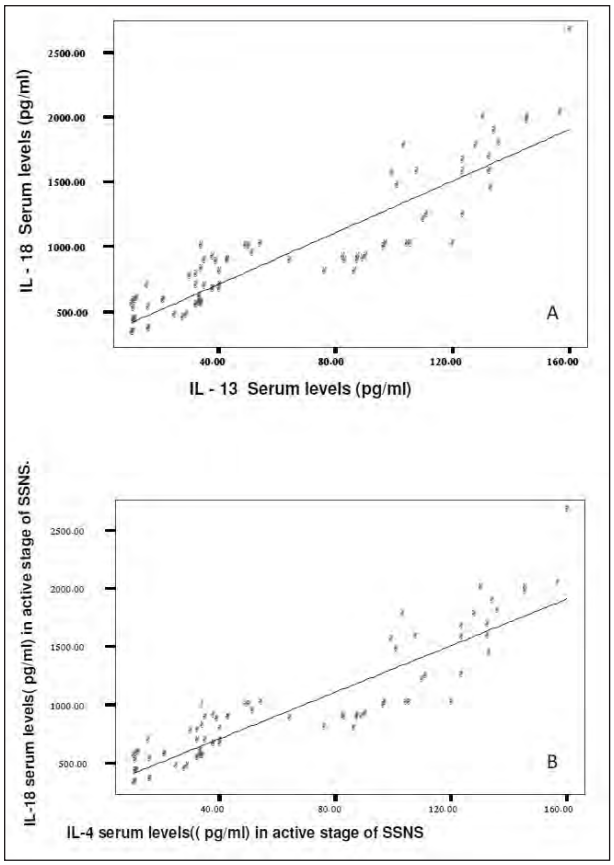


Figure-2: Correlation of serum IL-18 and IL-13 levels in children with steroid sensitive nephrotic syndrome (SSNS) in all stages (a) and in children with active disease (b)



al [9], and Cho *et al* [10], who reported an increased synthesis of IL-4 by stimulated peripheral mononuclear cells in patients with active SSNS. Also the results of the current study are in agreement with Printza *et al* [24] Kang *et al* [29], and Lama *et al* [30]. On the other hand, Cheung *et al* [27] did not report any increase in the percentage of IL-4 producing T cells.

Further support to the shift to type-2 immune response in our children with active SSNS is demonstrated by the increase of IL-13 serum levels in all our patients with SSNS. The results of this study are in accordance with pervious published studies [27, 19]. Yap *et al* [19] demonstrated an elevated expression of IL-13 mRNA using a semi-quantitative reverse transcriptase PCR technique. An experimental study on rat by Lai *et al* [31] demonstrated the occurrence of podocyte injury, inducing a minimal-change like nephropathy with the over-expression of IL-13. Recently, Printza *et al* [24] reported that serum IL-13 levels were significantly higher in the active stage of SSNS compared with the two remission phases and that although IL-13 levels were even lower than in the active stage, they remained elevated during both remission phases compared with the controls.

Our results highlight an important finding of increased levels of IL-18 in all disease stages, and particularly in the active stage of SSNS compared with the controls. This elevation should be greatly emphasized considering that IL-18 is a unique cytokine that can trigger both type-1 and type-2 immune responses depending on the predominant cytokines [2]. Earlier in the literature IL-18 was shown to be an IFN-γ trigger, which plays a critical role in the host defenses [32]. Recently, IL-18 has been postulated to induce IL-13 and/or IL-4 production by NK cells, mast cells and basophils [31, 32]. IL-18 is currently incriminated to play a potent role in various pathological conditions. The available data support its involvement in various diseases such as insulin dependent diabetes

mellitus, rheumatoid arthritis, Chron's disease and atopy.

In collaboration with IL-12, IL-18 stimulates Th1-mediated immune responses, which play a critical role in the host defense against infection with intracellular microbes through the induction of IFN-gamma. However, the overproduction of IL-12 and IL-18 induces severe inflammatory disorders, suggesting that IL-18 is a potent pro-inflammatory cytokine that has pathophysiological roles in several inflammatory conditions [2]. IL-18 mRNA is expressed in a wide range of cells including Kupffer cells, macrophages, T cells, B cells, dendritic cells, osteoblasts, keratinocytes, astrocytes, and microglia. Thus, the pathophysiological role of IL-18 has been extensively tested in the organs that contain these cells. Somewhat surprisingly, IL-18 alone can stimulate Th2 cytokine production as well as allergic inflammation. Therefore, the functions of IL-18 in vivo are very heterogeneous and complicated. In principle, IL-18 enhances the IL-12-driven Th1 immune responses, but it can also stimulate Th2 immune responses in the absence of IL-12 [31].

Printza *et al* [24] showed that in children with SSNS IL-18 levels were elevated in the active stage of the disease and that there was a positive correlation between IL-18 production and disease activity. Our results also demonstrated that in SSNS, IL-18 was significantly correlated with IL-4 and IL-13 which are type-2 cytokines.

Conclusion

In conclusion, the current study reports that during the active stage of SSNS the balance is tipped in favor of type-2 cytokine pattern, and that apparently IL-18 is correlated with these type-2 cytokines.

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